Virulence and Biofilm Potential of Bacillus amyloliquefaciens from Ultra-High Temperature Pasteurized Milk

An Honors Thesis (HONR 499)

By

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ABSTRACT

Bacillus amyloliquefaciens bacteria present in ultra-high temperature (UHT) pasteurized organic dairy milk may harbor virulence determinants, express toxins, and produce biofilms in a model food system. This project aimed to characterize *B. amyloliquefaciens*' virulence potential in comparison to *Bacillus cereus* ATCC14579, a well-established toxigenic type strain. Pure cultures of *Bacillus* spp. isolated from UHT pasteurized milk were identified to the species level using fatty acid profiling and rDNA sequencing. Confirmation of the presence of *plcR* and *codY* regulator genes and *nheA* and *hblC* enterotoxin genes was done using real-time PCR. RNA isolation from cultures grown in a model milk system was completed at predetermined time points from a growth curve, and target gene mRNA was amplified with real-time NASBA using a bioMerieux kit and transcript-specific primers to assess relative expression levels for each gene target. Biofilm production was monitored over 12 days in a model milk system on glass, stainless steel, and waxed paperboard, and the chemical composition of the biofilms was analyzed using complex carbohydrate analyses. We expect the rate of biofilm formation and composition to vary according to the type of solid support on which the biofilm forms. For real-time NASBA expression studies, no significant difference was measured between *B. amyloliquefaciens* and *B. cereus* ATCC14579 in mean cycle threshold values (relative expression levels) of 16s rRNA (p=0.249), *plcR* (p=0.188), *codY* (p=0.465), and *hblC* (p=0.148). There is a significant difference between *nheA* values (p<0.05); The significance of this project will be to determine if parameters for shipment, storage, and shelf life of UHT organic milk should be reassessed, in light of biofilm and virulence gene expression potential of this previously uncharacterized *B. amyloliquefaciens* strain.

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